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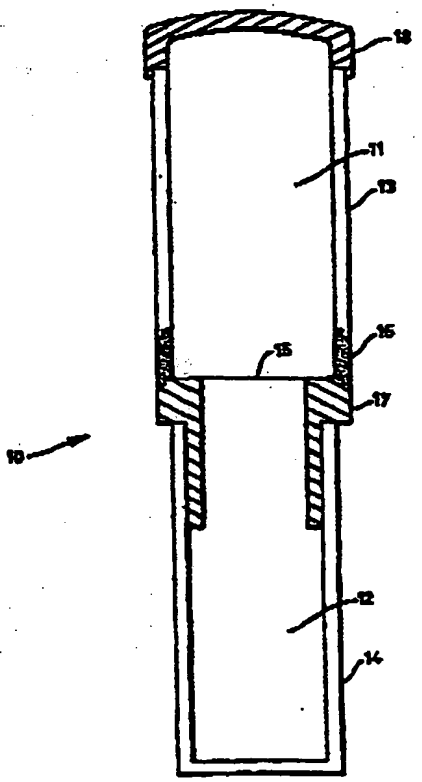
(54) Title: APPARATUS FOR ISOLATION AND PURIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS

(57) Abstract

A device, system, and method for isolating and purifying biologically active compounds. The inventive method and device utilize a centrifugal filtration device (10) employing a membrane (15) having ligands that preferentially bind a biologically active compound.



APPARATUS FOR ISOLATION AND PURIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS

FIELD OF THE INVENTION

This invention relates to an apparatus for isolating and purifying biologically active compounds, the use of which provides faster and more sensitive analyses than previously available.

BACKGROUND OF THE INVENTION

It has long been desired to purify and characterize biologically active compounds from their native fluids, e.g., blood serum, as well as cellular (mammalian, microbiological and other) extracts, lysates, enzymatic digests, bioreactors and cell cultures. Examples of such compounds are enzymes, proteins, drugs, peptides, nucleic acids, antigens, antibodies and complexes of such compounds, e.g., immune complexes (i.e., antigen-antibody pairs).

Existing devices for affinity purification use gels, hardened gel beads, silica beads and other matrices. All these devices require positive pressure to force the liquid through the stationary phase, therefore necessitating the use of pumps and liquid chromatographic instruments. These procedures require more time, preparation and skilled attention to obtain good results than is desirable. Further, the simultaneous running of large numbers of samples is not possible.

One practical and extensively used purification is that of murine monoclonal antibodies from ascites fluid and/or hybridoma cell cultures. In research monoclonal antibodies are used for the isolation of biologically active compounds from cellular extracts and other applications such as characterization of isolates. In other instances they are used for identification and diagnostic purposes. Furthermore, quick and small aliquot sample preparations are required for analysis and qualification of the monoclonal antibodies before the researcher scales up the purification to larger quantities of antibody from the cultures and/or ascites fluids. Research laboratories spend a disproportionate amount of time and effort for the above-described processes with presently-available technologies of affinity purification, using bacterial extracts such as staphylococcal protein A as the ligand on affinity matrices mentioned above.

Another example is the isolation of immune complexes from patient serum samples and consequent purification of the antigen from the isolated immune complex. A practical method for isolating rare biologically active compounds is immunoprecipitation, in which *in vitro* immune complexes are produced in complex

mixtures, followed by isolation with protein A, then purification of the antigen and antibody components using ultrafiltration.

Tests for immune complexes should be sensitive, specific, and reproducible. It has been noted that by dissociating immune complexes through acidification and microfiltration of the patient's sera, the incidence and titer of these antibodies have been significantly augmented. Previous methodology used a standard ultrafiltration chamber fitted with a 100,000 MW cutoff membrane. A drawback of this technique, however, is that it takes between 48 and 72 hours to process a single serum specimen.

In conventional affinity membrane applications it is important to control the rate of fluid passage through the membrane to allow the biologically active compound to bind. This is difficult to do, however, if using conventional means to move the fluid through the membrane, such as pressure or vacuum, and highly impractical to make inexpensive, disposable devices using the pressure or vacuum design principle. The equipment used employing these conventional techniques also requires more skill and effort to perform the analyses than is desirable. Also, such existing methods are not easily adaptable for multi-sample processing, or to automation.

It is therefore an object of this invention to provide a simple, reproducible and inexpensive method for isolating and purifying biologically active compounds from fluid containing them (and an device for doing so) that is faster than prior methods and devices, but with no loss in sensitivity or yield, and one which can further be adapted to multi-sample processing or automation.

SUMMARY OF THE INVENTION

The present invention relates to a centrifugal device for preferentially removing biologically active compounds from a fluid, comprising at least one capture unit comprising a) an upper chamber for receiving fluid to be treated; b) a lower chamber in fluid communication with the upper chamber, for receiving filtrate; and c) a porous capture membrane layer disposed between the upper and lower chambers, the membrane layer having ligands for preferentially binding a biologically active compound.

The invention further relates to a method of purifying biologically active compounds comprising the steps of a) providing a centrifugal device for preferentially removing biologically active compounds from a fluid, the device comprising at least one capture unit comprising an upper chamber for receiving fluid to be treated; a lower chamber in fluid communication with the upper chamber, for receiving filtrate; and a porous capture membrane layer disposed between the upper and lower chambers, the membrane layer having ligands for preferentially binding a biologically active compound; b) adding a fluid sample to the upper chamber and centrifuging the capture

device to produce a filtrate; c) removing the filtrate from the lower chamber; d) treating the porous capture membrane layer with an eluting solvent so as to elute the biologically active compound off the porous membrane layer; and e) centrifuging the capture device to produce an eluent.

DESCRIPTION OF THE DRAWINGS

Figure 1 depicts an embodiment of a device according to the invention, in longitudinal cross-section.

DESCRIPTION OF THE INVENTION

Our invention relates to devices, systems and methods for purifying biologically active compounds. An exemplary embodiment of our device is shown in longitudinal cross-section in FIG. 1. Device 10 (hereinafter termed "centrifugal device") comprises upper chamber 11, into which fluid sample to be processed is placed. Upper chamber 11 is defined by the substantially cylindrical wall 13. At the upper edge of wall 13 is found cap 18, which protects the contents of upper chamber 11. Cap 18 can be, e.g., snap fit on or attached by a screw thread. At the lower edge of wall 13 is a capture membrane locking sleeve 16, which securely sandwiches capture membrane layer 15 between the interlocking locking sleeve 16 and chamber end cap 17. Capture membrane layer 15 thus forms a semi-permeable barrier between upper chamber 11 and lower chamber 12, which has a closed end to form a fluid receptacle. The membrane may also be secured by other conventional means, e.g., by using an o-ring.

Chamber end cap 17 is designed to frictionally fit into cup 14, which defines lower chamber 12. In this manner the entire unit may be placed into a centrifuge shield for centrifugation. When device 10 is spun in the centrifuge, fluid sample is pulled from upper chamber 11 to lower chamber 12 through capture membrane layer 15. The filtrate thus produced in cup 14 may then be decanted by detaching the cup.

The capture membrane layer is a porous membrane having ligands for preferentially binding a biologically active compound. (The word "membrane" as used herein is meant to not only refer to true "membranes", but to all forms of porous planar filter media, e.g., papers, etc.) The capture membrane material may be any material suited to this purpose; the membranes, and means for coupling ligands to ("activating") the membranes are well-known, for example, cellulosic, nylon, and polysulfone membrane materials. The nature of the membrane is important inasmuch as it should be fairly inert with respect to the solvents used, and the biologically active compounds of interest, and must allow for the ligand attachment. The pore size of the membrane material is dependent on the particular biologically active compound sought to be isolated, but, in general, the average pore size must be at least large enough to allow unwanted components to pass through the membrane layer. Membrane materials that

we have found useful, while not intended to be limiting, may be found in Table I. Microfiltration and ultrafiltration ("UF") type membranes are preferred, with microfiltration membranes particularly preferred.

TABLE I

Membrane Name*	Membrane Type	MWCO†	Classification
YC05	Cellulosic	500	Ultrafiltration
YM1	"	1000	"
YM3	"	3000	"
YM10	"	10000	"
YM30	"	30000	"
YM100	"	100000	"
PM10	Polysulfone	10000	"
PM30	"	30000	"
XM50	DYNEL™	50000	"
XM300	"	300000	"
GLS.2	Polysulfone	0.2μ‡	Microfiltration
GLS.45	"	0.45μ‡	"
"Bio-38"	Cellulosic	5μ‡	"

*AMICON, INC., BEVERLY, MASS. †molecular weight cutoff ‡avg. pore size

The more important factor in selecting a capture membrane layer is the biologically active compounds themselves, because the nature of the compounds will most determine a) the particular ligand to be attached to the membrane and b) the membrane material to be used. The choice of ligand is more important because the complex will not bind to the membrane otherwise. Exemplary ligands, are shown in Table II. Other ligands, or combinations thereof, may be arrived at by those of ordinary skill in the art.

TABLE II

Ligand
protein A
protein G
lectins
antibodies
antigens
chiral moieties
strep-avidin
biological complexes
α-chymotrypsin
nucleic acids

The capture membrane layer may preferably comprise one or more sheets of membrane, one laid on top of the other. This is particularly advantageous, because several sheets of membrane laid on top of each other increase the capacity for the biologically active compound on the membrane, and enhance mass transfer and opportunity for binding of the biologically active compound to the capture membrane layer.

The isolation or purification of biologically active compounds according to our invention may be carried out as follows. Fluid, for example, blood serum, containing the biologically active compound(s) of interest is first placed into the upper chamber of our centrifugal device generally described above. The device is then placed into a centrifuge and spun for a time and speed sufficient to move the fluid component from the upper chamber through the capture membrane and into the lower chamber, thus producing a filtrate. The biologically active compound(s) will preferentially bind to the membrane. The optimal centrifuge time and speed may be chosen without much effort; at too high a speed, it can be anticipated that some of the biologically active compounds of interest, passing through the membrane too quickly, will not bind and will be lost in the filtrate. It is therefore advisable to use a slower speed initially and gradually work the speed up in subsequent runs if desired. The centrifugal device only needs to be spun long enough to remove liquid from the upper chamber; when employing an ultrafiltration membrane, however, the membrane must be left moistened. Temperature-sensitive samples may be processed in a temperature controlled centrifuge environment if necessary.

The device may then be removed and the capture membrane preferably washed to remove unbound compounds, with one or several volumes (each wash volume preferably roughly equal to the fluid capacity of the upper chamber) of, e.g., DI water or a neutral buffer solution, with centrifugation following each wash. The filtrate and washes may be saved or discarded as necessary by detaching the cup on the bottom of the unit, decanting the fluid and reattaching the cup.

After this, the capture membrane is treated with an eluting solvent to elute the biologically active compounds off the membrane. The eluting solvent chosen is dependent on the nature of the ligand and ligate, and the selection of the solvent may be done by those of ordinary skill in the art. The capture device is centrifuged again; the biologically active compound is eluted off the membrane and passes through the filter into the lower chamber as the filtrate.

It should be noted that the embodiment of the device described above may be reconfigured into many different embodiments and combinations. Likewise, the method of the invention may be employed in combination with other analytical tools to achieve a particular end. For example, the device described above could be configured from a single tube-type apparatus into a unitary plate containing an 8x12 array of "upper chamber" wells (i.e., in the standard 96 well plate configuration) in order to use the variety of equipment and methodologies, e.g., centrifuges, plate readers, that support the format. Continuing further with this example, this "capture plate" would snap onto or otherwise cooperatively attach to a standard 96 well plate to allow individual centrifugal isolation or purification as previously described herein. Furthermore, capture plates having different capture membrane layers specific for different biologically active compounds could be stacked on top of one another to isolate each biologically active compound from the other, thus reducing several separations to one. In another embodiment along these lines, the bottommost capture plate could be made having capture membranes in each well which may be used for membrane-bound ELISA assays.

The device described in FIG. 1 above could also be configured to stack "upper chamber" units in one "single-position" device to isolate different biologically active compounds from a fluid in one step, as in the 96 well capture plate just described. Such a capture device would have a greater capacity for each biologically active compound, and, with an appropriate variety of capture membranes, would allow the researcher to custom configure a device for his or her own requirements. The advantage that all these 'stackable' embodiments of our invention share over current affinity membrane methods, i.e., vacuum, positive pressure or gravity pull through the membrane, is that the force pulling the sample through the membrane will be equal in each "upper

chamber", which is critical for ensuring equality of mass transfer, and thus consistency of biologically active compound retention, over each capture membrane in the device.

One further convenient advantage of the embodiment shown in FIG. 1 is that elution of the biologically active compound(s) retained on the membrane may be done by inverting the unit, adding eluting solvent to the receptacle formed by the cylindrical wall of chamber end cap 17, and centrifuging the inverted unit so as to elute the retained compound into the cap 19.

What Is Claimed Is:

1. A centrifugal device for preferentially removing biologically active compounds from a fluid, comprising at least one capture unit comprising
   a) an upper chamber for receiving fluid to be treated;
   b) a lower chamber in fluid communication with said upper chamber, for receiving filtrate; and
   c) a porous capture membrane layer disposed between said upper and lower chambers, said membrane layer having ligands for preferentially binding a biologically active compound.

2. The device of claim 1 wherein said capture membrane layer comprises a plurality of membranes in a stack.

3. The device of claim 1 wherein said capture membrane layer is of the microfiltration type.

4. The device of claim 1 wherein said capture membrane layer is selected from the group consisting of cellulosic, nylon, and polysulfone membrane materials.

5. The device of claim 1 wherein said ligands are selected from the group consisting of protein A, protein G, lectins, antibodies, antigens, chiral moieties, strep-avidin, biological complexes, α-chymotrypsin, nucleic acids, and combinations thereof.

6. The device of claim 1 comprising a plurality of said capture units.

7. The device of claim 6 wherein said capture units are laid out in a planar 8x12 array.

8. The device of claim 1 wherein said lower chamber terminates in a closed end so as to form a receptacle.

9. A method of purifying biologically active compounds comprising the steps of

a) providing a centrifugal device for preferentially removing biologically active compounds from a fluid, said device comprising at least one capture unit comprising 1) an upper chamber for receiving fluid to be treated; 2) a lower chamber in fluid communication with said upper chamber, for receiving filtrate; and 3) a porous capture membrane layer disposed between said upper and lower chambers, said membrane layer having ligands for preferentially binding a biologically active compound;

b) adding a fluid sample to said upper chamber and centrifuging said centrifugal device to produce a filtrate;

c) removing said filtrate from said lower chamber;

d) treating said porous capture membrane layer with an eluting solvent so as to elute said biologically active compound off said porous membrane layer; and

e) centrifuging said centrifugal device to produce an eluent.

10. The method of claim 9 wherein said membrane layer comprises a plurality of membranes in a stack.

11. The method of claim 9 wherein said membrane layer is of the microfiltration type.

12. The method of claim 9 wherein said capture membrane layer is selected from the group consisting of cellulosic, nylon, and polysulfone membrane materials.

13. The method of claim 9 wherein said ligands are selected from the group consisting of protein A, protein G, lectins, antibodies, antigens, chiral moieties, strep-avidin, biological complexes, α-chymotrypsin, nucleic acids, and combinations thereof.

14. The method of claim 9 wherein said device comprises a plurality of said capture units.

15. The method of claim 14 wherein said capture units are laid out in a planar 8x12 array.

16. A system for preferentially removing biologically active compounds from a fluid, comprising

a) a centrifugal device comprising at least one capture unit comprising
- an upper chamber for receiving fluid to be treated;
- a lower chamber in fluid communication with said upper chamber, for receiving filtrate; and
- a porous membrane layer disposed between said upper and lower chambers, said membrane layer having ligands for preferentially binding a biologically active compound;

b) and a centrifuge.

17. The system of claim 16 wherein said capture membrane layer is selected from the group consisting of cellulosic, nylon, and polysulfone membrane materials.

18. The system of claim 16 wherein said ligands are selected from the group consisting of protein A, protein G, lectins, antibodies, antigens, chiral moieties, strep-avidin, biological complexes, α-chymotrypsin, nucleic acids, and combinations thereof.

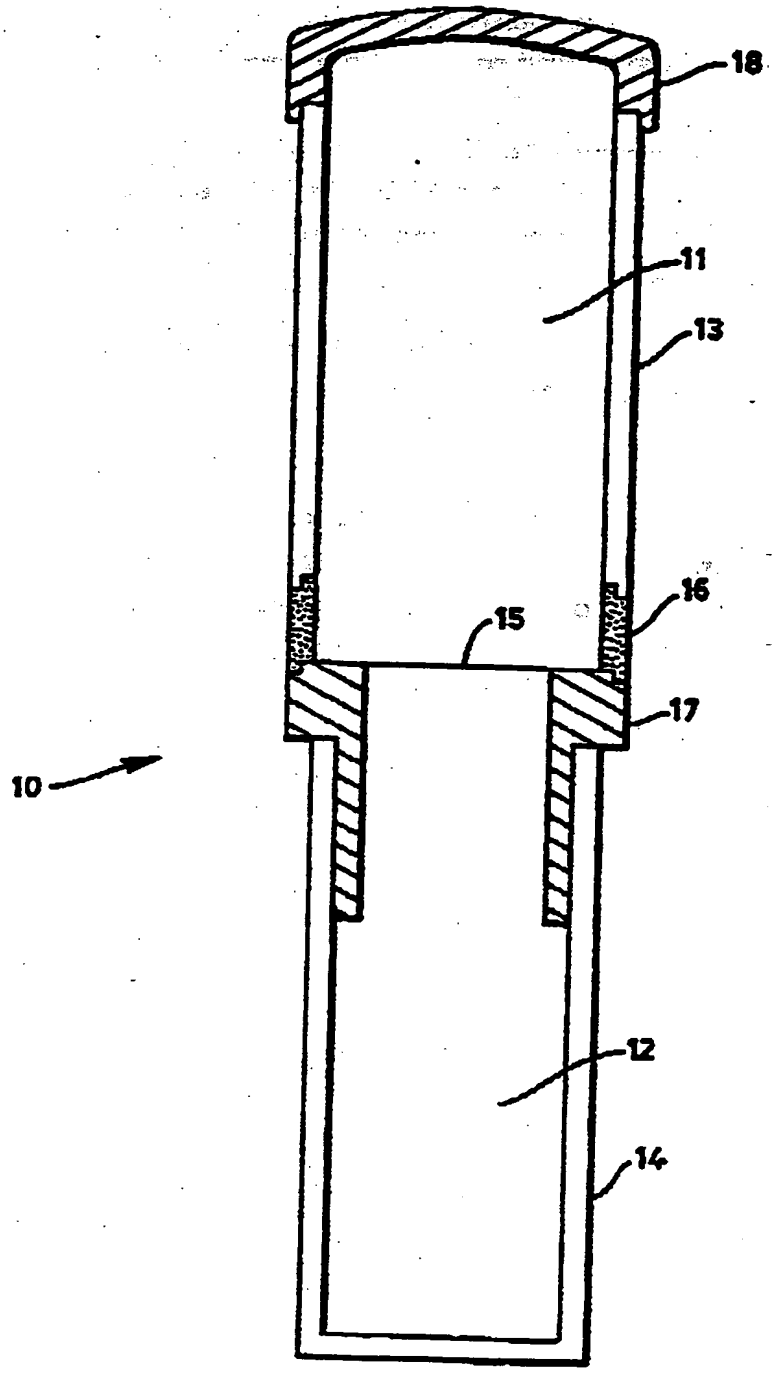


FIG. 1

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01L3/14 B04B5/04 G01N33/543 B01D61/00 C07K1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 07648 (FMC CORP.) 30 May 1991 see page 1, line 6 - line 18 see page 7, line 1 - line 35	1,3-5,8, 9,11-13, 16-18
Y	see page 9, line 26 - page 10, line 10; figure see page 23, line 14 - page 24, line 24	6,7,14, 15
Y	EP,A,0 505 118 (MINNESOTA MINING) 23 September 1992 see column 2, line 42 - column 3, line 22 see column 5, line 47 - line 55; claim 6	6,7,14, 15
A	EP,A,0 569 115 (GENERAL ATOMICS) 10 November 1993 see column 4, line 27 - line 44; figures	6,7, 14-16

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 July 1995	04.08.95

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	EP,A,0 594 506 (PASTEUR SANOFI) 27 April 1994 see page 3, column 14 - column 20; claim 1	9

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